A Further Study of the Mitotic Spindle in the Spermatocytes of Forficula auricularia.

By

C. F. U. Meek.

With Plates 1–2.

Introduction.

At the beginning of 1913 I stated that the lengths of the mitotic spindle at the conclusion of the primary and secondary spermatocyte metaphases of Forficula auricularia seemed to be constants, and that the ratio between these lengths was almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other. The primary spermatocyte cell divides to form two secondary spermatocytes; and, since no period of growth seems to separate their mitoses, the volume of the primary spermatocyte cell in the metaphase is presumably equal to twice that of the daughter secondary spermatocyte: connection was therefore suggested between the spindle-length and cell-volume at this stage.

I then measured spindle-lengths at the conclusion of the spermatocyte metaphases of Helix pomatia; the lengths again seemed to be constants, and the ratio between them was found to be identical with the second ratio mentioned above. Moreover, independent investigations made at this time by von Winiwarter showed the same phenomenon in the spermatocyte mitoses of man.

I have since published photo-micrographs verifying my earlier camera lucida drawings of the spindles of Forficula

auricularia and Helix pomatia; but, in new preparations of the former, have observed primary spermatocyte spindle-lengths that are excessive, and that consequently do not accord with the ratio previously found. Four explanations were put forward to account for these lengths. "Firstly, the volume of these cells in the metaphase may vary, and our proposition may still be valid. In this case, however, various lengths will presumably be found at the conclusion of the secondary spermatocyte metaphase; and I have not observed such lengths. Secondly, the daughter-chromosomes may remain apposed to one another in the equatorial plane for a considerable time after constriction is complete; if centrosome divergence continues during this period, the various and excessive lengths may be explained. This, however, cannot always occur; for, in this organism, I have found and drawn primary spermatocyte cells in which the daughter-chromosomes have begun to move towards the poles when the spindle-length is only slightly greater than that estimated for the conclusion of the metaphase. Thirdly, our proposition may require modification in that the length of the spindle may be affected by the shape of the cell. My original measurements in Forficula and Helix were made from cells that were approximately spherical, and this may explain the constant lengths observed. When, however, cells are closely packed together in a cyst, the spherical form disappears, and if our modification is valid, the spindle-length will vary with the shape assumed. Lastly, the length of the spindle at this stage may be connected with neither the volume nor shape of the cell; and in this case our proposition is entirely disproved. If, however, this is so, why has the ratio in question been observed in Helix pomatia and man?"

We know little concerning the nature of the mitotic spindle, although it has been a subject of investigation for many years. We have found that it cannot be regarded as a figure formed entirely by the action of forces at its poles. I have since shown that its length at the conclusion of certain spermatogenetic metaphases cannot be correlated with the

volume of chromatin present; and, if we can prove that the length at this stage is or is not determined by the volume of the cell, we shall have succeeded in establishing another proposition.

In the circumstances, I now intend to investigate further the spermatocyte spindles of Forficula auricularia. That the length at the conclusion of the primary spermatocyte metaphase is not a constant has already been proved; but the results of this study may constitute new data affecting the proposition that I have put forward.

Material and Methods.

The material, which was obtained in July and August, was preserved in Flemming's strong chromo-aceto-osmic acid fluid. The testes remained in the fixative for twenty-four hours, and, after being thoroughly washed in running water and passed through successive strengths of alcohol, were cleared in xylol and embedded in paraffin. Sections were cut 8 μ thick with a Cambridge rocking microtome.

All sections were stained on the slide. The slides were placed for four to six hours in an aqueous solution of ferric alum, and were then stained for twelve to fifteen hours in Heidenhain's iron-hæmatoxylin. In certain cases they were first stained for ten minutes in eosin.

The preparations were studied with a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1·30, and the various compensating oculars. The light was obtained from an inverted incandescent gas-burner, and was passed through a Watson holoscopic oil-immersion substage condenser. All photo-micrographs shown were made with a Zeiss camera, the apochromatic objective mentioned above, and compensating ocular No. 4. The camera extension was 50 cm. in the case of all photographs of individual cells, and 25 cm. in the case of photographs of cysts. The magnification was estimated with a stage micrometer graduated to read one hundredth part of a millimetre, and photographs of this scale are in-

cluded in the plates. Moreover, in order to minimize error, all measurements have been independently checked by my assistant, Mr. T. R. Goddard.

The Length of the Spindle in the Primary Spermatocyte Metaphase and Early Anaphase.

Fig. 1, Pl. 1, shows a section of two cysts containing primary spermatocyte cells, stained with iron-hæmatoxylin. Two polar views are seen, in which the reduced number of chromosomes can be counted; and, since both metaphases and telophases are observed, the cells are in various stages of mitosis. Fig. 2 shows a section of a cyst of these cells, taken from the testes of another specimen and stained with iron-hæmatoxylin and eosin; the cells in this section are seen to be in the metaphase. Fig. 46, Pl. 2, shows the magnification of these two photo-micrographs.

Figs. 7 and 8, Pl. 2, are polar views of primary spermatocyte cells in the metaphase, and show twelve chromosomes; the latter figure has been taken from the section of the cysts shown in fig. 1. These and all following photographs of cells have been made at a magnification approximately equal to twice that of figs. 1 to 6, Pl. 1, and a photograph of the divisions of the stage micrometer is given in fig. 47, Pl. 2.

Figs. 9 to 26 inclusive are lateral views of primary spermatocyte cells in the metaphase, and the chromosomes are seen to be constricting in the equatorial plane. Figs. 10 and 17 also show polar views, which are similar to those of figs. 7 and 8. Figs. 15, 18, and 23 have been taken from the section of cysts shown in fig. 1, in which photograph the first two figures are seen in focus. Fig. 25 belongs to the section and cyst shown in fig. 2. The lengths of these eighteen spindles, estimated from the magnification, are given in the following table[1]:

[1] In this paper I am not attempting to express spindle-lengths in terms smaller than one quarter of a micromillimetre.

Fig. 9	11·25 μ	Fig. 18	14·0 μ
„ 10	11·25 μ	„ 19	14·5 μ
„ 11	11·5 μ	„ 20	14·75 μ
„ 12	12·0 μ	„ 21	16·0 μ
„ 13	12·25 μ	„ 22	16·25 μ
„ 14	12·5 μ	„ 23	16·5 μ
„ 15	12·75 μ	„ 24	16·5 μ
„ 16	12·75 μ	„ 25	16·75 μ
„ 17	13·25 μ	„ 26	17·5 μ

Now these figures prove that the length of the spindle may vary considerably at the stage immediately preceding the conclusion of the metaphase. The spindles shown in the photographs cannot be regarded as abnormal, and their lengths, which constitute an approximately graded series, exceed in every case that originally observed by me at the slightly later stage, when constriction of the chromosome is completed.

I said in my last paper that I had found various and excessive spindle-lengths at the latter stage; and in the course of the present research I have again observed such lengths, e. g. 12·0, 13·5, 15·25, 15·75, 16·25, 16·5, 17·0 and 18·0 μ. In the circumstances, we must realise that the length of the spindle in this mitosis is not a constant at either of the stages mentioned. Moreover, I have now found these excessive lengths in certain cysts of my older material; we have, therefore, no reason for supposing that individuals of the species differ in this respect.

We will now consider the early anaphase. Figs. 27 and 28 are lateral views of primary spermatocyte cells at this stage, and the spindle-lengths, estimated from the magnification, are respectively 11·25 and 11·5 μ. These lengths are smaller than those seen in the cells represented by figs. 12 to 26 inclusive, and the figures consequently prove that the length of the spindle when the daughter-chromosomes have begun to move towards the two poles may be smaller—and sometimes considerably smaller—than the length before constriction of the chromosomes is completed. Furthermore, I have found

cells in which the distance between the daughter-chromosomes is approximately the same as that in figs. 27 and 28, whereas the spindle-length greatly exceeds 11·5 μ. The amount of divergence of the daughter-chromosomes is therefore not invariably proportional to the length of the spindle in the anaphase of this mitosis.

The Length of the Spindle in the Secondary Spermatocyte Metaphase and Early Anaphase.

Figs. 3 and 4, Pl. 1, show sections of cysts of secondary spermatocyte cells in various stages of mitosis; these sections were stained with iron-hæmatoxylin. Figs. 5 and 6 show sections of similar cysts, taken from the testes of another specimen and stained with iron-hæmatoxylin and eosin; the cells in these figures are seen to be in the metaphase. These four photographs have been made at the same magnification as figs. 1 and 2.

Figs. 29, 30 and 31, Pl. 2, are polar views of secondary spermatocyte cells in the metaphase, and twelve chromosomes can be counted in each. Fig. 29 was taken from the section of the cyst shown in fig. 3, Pl. 1, and the figure is seen to be in focus in the latter photograph.

Figs. 32 to 42 inclusive are lateral views of these cells in the metaphase, before constriction of the chromosomes is completed. Figs. 36 and 37 belong to the section of the cyst shown in fig. 3; figs. 41 and 42 belong to that shown in fig. 5. Fig. 40 was taken from the section and cyst shown in fig. 6, and fig. 32 belongs to the same section and cyst as fig. 30. The lengths of the spindle in these eleven cells, estimated from the magnification, are given in the table below:

Fig. 32 . . .	6·75 μ	Fig. 38 . . .	8·75 μ
,, 33 . . .	7·0 μ	,, 39 . . .	9·0 μ
,, 34 . . .	7·0 μ	,, 40 . . .	10·25 μ
,, 35 . . .	7·5 μ	,, 41 . . .	10·25 μ
,, 36 . . .	7·5 μ	,, 42 . . .	11·25 μ
,, 37 . . .	7·5 μ		

Now, it is evident from these measurements that the length of the spindle is not a constant at the stage immediately preceding the conclusion of the secondary spermatocyte metaphase; the lengths that I originally observed at this stage and at the conclusion of the metaphase were respectively 7·8 and 8·1 μ, and the lengths shown in the table are in every case greater or smaller than these. This presupposes various lengths at the conclusion of the metaphase, and I have now found such lengths both in my new material and in certain cysts of my older preparations. The discovery of these various lengths is important; for I stated in my last paper that I had not found them in this cell generation.

Figs. 43, 44 and 45 are lateral views of these cells in the anaphase. Fig. 43 was taken from the section of the cyst shown in fig. 3; figs. 44 and 45 were taken from that shown in fig. 4. The lengths of the spindle in these cells are respectively 8·25, 8·75, and 10·75 μ; and, since we have observed greater lengths in cells in which constriction of the chromosomes is not completed, the results are similar to those already obtained from the study of the primary spermatocyte mitosis. Moreover, I have found cells in which the distance between the daughter-chromosomes is approximately the same as that in figs. 43 and 44, whereas the spindle-lengths considerably exceed 8·75 μ; the length of the spindle cannot therefore be proportional to the amount of chromosome divergence in the early anaphase of the secondary spermatocyte cells.

The Four Explanations previously put forward.

Having proved that the spindle-length in these two mitoses is not a constant at either the conclusion of the metaphase or the stages immediately preceding and following the conclusion, we can consider the explanations that were put forward when I discovered various and excessive lengths in the primary spermatocyte cells. Of these explanations, three imply the

validity of my original proposition, or of a modification of that proposition; the fourth implies its entire refutation.

Firstly, I suggested that the volumes of the primary spermatocyte cells vary in the metaphase. Secondly, I suggested that the daughter-chromosomes remain opposed to one another in the equatorial plane for a considerable time after constriction is completed; if centrosome divergence continues during this period, the various and excessive lengths may be explained. Both explanations assume that the length of the spindle is proportional to the volume of the cell at the moment when constriction of the chromosomes is completed. We will deal first with the former.

We know that a long period of growth separates the last spermatogonial mitosis and that of the primary spermatocytes, but we do not know that the consequent increase of volume of the latter cells is the same in all cases. Furthermore, we have no proof that the volumes of the parent spermatogonia are constant, and therefore cannot say that all primary spermatocytes are identical in volume at the moment of their formation. The objection to this explanation was that the spindle-length must presumably vary at the conclusion of the secondary spermatocyte metaphase, whereas it seemed to be constant. This objection, however, has now been removed; for the further study of these cells has revealed the existence of various lengths at this stage.

Now, the photographs of individual cells given in Pl. 1 show marked differences between the areas enclosed by the cell outlines. We cannot, however, infer from this that the volumes vary; for the cells are seen in only two dimensions. If we wish to prove that the volumes vary, we must study the cells in various horizontal planes; and, since we cannot hope to form more than a rough estimate of the volume in any individual case, it will be advisable to compare cells in which the volumes in corresponding planes differ considerably. Moreover, in dealing with this explanation we must be careful to compare cells to which the second explanation cannot apply; otherwise no trustworthy conclusion can

be drawn from our results. The conditions mentioned above are fulfilled in the cells shown in figs. 21 and 25 of the plate. We will first consider the former.

The area enclosed by the outline of this cell in the horizontal plane through the centrosomes is approximately 430 sq. μ, and camera lucida drawings of the cell-outline at various vertical distances from this plane prove that the area enclosed does not vary appreciably throughout the section. The greatest vertical distance between any two planes studied is 8 μ, i. e. the thickness of the section; and, since the mean area may be said to be 430 sq. μ, the volume of the portion measured must be approximately 3440 c. μ. Unfortunately, I have not been able to follow the contour further; for I have failed to identify the cell in the sections immediately preceding and following that in which the spindle lies. We have reason for supposing that a large portion lies outside this section; but, whether this is or is not so, the minimum volume of the cell cannot be less than 3440 c. μ.

We will now consider the cell shown in fig. 25. I have made numerous photo-micrographs at various vertical intervals of the cyst in which this cell lies; the horizontal planes represented extend through three consecutive sections, and cover a vertical distance of 20 μ. A comparison of these photographs shows that the maximum area enclosed by the cell-outline is found in the horizontal plane that passes through the centrosomes, and that the cell cannot extend for a distance greater than 8 μ above and below this plane. The maximum area enclosed is approximately 160 sq. μ; and, since the depth of the cell cannot exceed 16 μ, the volume must be less than 2560 c. μ. But we have already proved that the volume of the cell shown in fig. 21 is at least 3440 c. μ: it is therefore evident that the volumes of the primary spermatocyte cells vary in the metaphase.

We must now compare the lengths of the spindle in these two cells. A glance at the table on page 5 shows that the smaller spindle-length is found in the larger cell; consequently, the length of the spindle at the stage shown cannot

be proportional to the volume of the cell. A study of the equatorial plates of these two figures at a high magnification shows respectively only one and two chromosomes that have not completed constriction. For our present purpose, therefore, the stage shown may justifiably be identified with the conclusion of the metaphase; and, since the second explanation cannot be applied in this case, we must realise that the length of the spindle at the conclusion of the primary spermatocyte metaphase is not proportional to the volume of the cell. But the first and second explanations were put forward to support the proposition that the spindle-length at this stage is proportional to the volume of the cell: neither explanation need therefore be discussed further.

Two explanations remain to be considered. One implies a modification of my original proposition in that the length of the spindle at the conclusion of the metaphase is said to depend upon both the volume and the shape of the cell; the other denies that it is controlled by this combination. The explanations are accordingly antithetical, and proof of one must constitute disproof of the other.

The only satisfactory method of dealing with this question is to compare cells in which both factors are identical, or cells in which one factor differs; if the suggested connection is not to be disproved, we must find similar spindle-lengths in the former, and different spindle-lengths in the latter respectively. And, in either case, the cells compared must be spherical: a rough estimate may suffice to prove that cells of various shapes differ in volume; but we cannot hope for accuracy sufficient to prove that in certain cases the volumes of such cells are identical. Unfortunately, my material is unsuited to this investigation; for the cells are closely packed together in the cysts, and are consequently distorted. We must therefore defer consideration of the two remaining explanations until suitable material is available.

Conclusion.

The present research is now finished, and we have seen that the results obtained contradict the proposition put forward in my earlier paper upon this organism. I have carefully checked the measurements that led me to put forward this proposition, and have found them to be accurate; but the new evidence before us shows that it cannot be universal. In the circumstances, another negative proposition seems to have been established concerning the spindle. We have found that it is not a figure formed entirely by the action of forces at its poles; we have found that its length at the conclusion of the metaphase is not proportional to the volume of the chromatin; and we have now found that the length at this stage is not proportional to the volume of the cell. We must, however, remember that these negative propositions have been established for individual cases, and are therefore generalisations only in that their antitheses cannot be put forward as being invariably valid.

Furthermore, we have seen that the volumes of the primary spermatocyte cells of Forficula vary in the metaphase; and we have no reason for supposing that such variation is confined to this organism. In the circumstances, a comparison of the volumes of spermatocyte cells in different organisms cannot prove specific similarities or dissimilarities until we have satisfied ourselves that the volumes do not vary, or until we have ascertained the limits of variation in each case.

I hope to deal further with these questions in a subsequent paper. The spindle is a phenomenon of mitosis; and, if we can discover the factors that determine its length, we shall be one step nearer an understanding of its nature and the phenomena that are inseparably connected with it.

Summary.

(1) The length of the spindle at the stage immediately preceding the conclusion of the primary spermatocyte metaphase is not a constant.

(2) The length of the spindle at the conclusion of the primary spermatocyte metaphase is not a constant, and is sometimes smaller than that observed at the stage immediately preceding the conclusion.

(3) The length of the spindle in the early primary spermatocyte anaphase is not proportional to the amount of chromosome divergence, and is sometimes smaller than the lengths observed at the stages mentioned in (1) and (2).

(4) The volumes of the primary spermatocyte cells vary in the metaphase.

(5) The length of the spindle at the conclusion of the primary spermatocyte metaphase is not proportional to the volume of the cell.

(6) The length of the spindle at the stage immediately preceding the conclusion of the secondary spermatocyte metaphase is not a constant.

(7) The length of the spindle at the conclusion of the secondary spermatocyte metaphase is not a constant, and is sometimes smaller than that observed at the stage immediately preceding the conclusion.

(8) The length of the spindle in the early secondary spermatocyte anaphase is not proportional to the amount of chromosome divergence, and is sometimes smaller than the lengths observed at the stages mentioned in (6) and (7).

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Bibliography.

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EXPLANATION OF PLATES 1 AND 2,

Illustrating Mr. C. F. U. Meek's paper on "A Further Study of the Mitotic Spindle in the Spermatocytes of Forficula auricularia."

PLATE 1.

Fig. 1.—Section of cysts containing primary spermatocyte cells in various stages of mitosis. Section stained with iron-hæmatoxylin.

Fig. 2.—Section of cyst containing primary spermatocyte cells undergoing mitosis. Section stained with iron-hæmatoxylin and eosin.

Figs. 3 and 4.—Sections of cysts containing secondary spermatocyte cells in various stages of mitosis. Sections stained with iron-hæmatoxylin.

Figs. 5 and 6.—Ditto. Sections stained with iron-hæmatoxylin and eosin.

PLATE 2.

Fig. 7.—Polar view of primary spermatocyte cell in the metaphase, showing twelve chromosomes.

Fig. 8.—Ditto. (Taken from section of cyst shown in fig. 1.)

Figs. 9 and 10.—Lateral view of primary spermatocyte cell in the metaphase; the chromosomes are constricting. Length of spindle 11·25 μ.

Fig. 11.—Ditto. Length of spindle 11·5 μ.

Fig. 12.—Ditto. Length of spindle 12·0 μ.

Fig. 13.—Ditto. Length of spindle 12·25 μ.

Fig. 14.—Ditto. Length of spindle 12·5 μ.

Fig. 15.—Ditto. Length of spindle 12·75 μ. (Taken from section of cyst shown in fig. 1.)

Fig. 16.—Ditto. Length of spindle 12·75 μ.

Fig. 17.—Ditto. Length of spindle 13·25 μ.

Fig. 18.—Ditto. Length of spindle 14·0 μ. (Taken from section of cyst shown in fig. 1.)

Fig. 19.—Ditto. Length of spindle 14·5 μ.

Fig. 20.—Ditto. Length of spindle 14·75 μ.

Fig. 21.—Ditto. Length of spindle 16·0 μ.

Fig. 22.—Ditto. Length of spindle 16·25 μ.

Fig. 23.—Ditto. Length of spindle 16·5 μ. (Taken from section of cyst shown in fig. 1.)

Fig. 24.—Ditto. Length of spindle 16·5 μ.

Fig. 25.—Ditto. Length of spindle 16·75 μ. (Taken from section of cyst shown in fig. 2.)

Fig. 26.—Ditto. Length of spindle 17·5 μ.

Fig. 27.—Lateral view of primary spermatocyte cell in the early anaphase, showing divergence of daughter-chromosomes. Length of spindle 11·25 μ.

Fig. 28.—Ditto. Length of spindle 11·5 μ.

Fig. 29.—Polar view of secondary spermatocyte cell in the metaphase, showing twelve chromosomes. (Taken from section of cyst shown in fig. 3.)

Figs. 30 and 31.—Ditto.

Fig. 32.—Lateral view of secondary spermatocyte cell in the metaphase; the chromosomes have not completed constriction. Length of spindle 6·75 μ.

Figs. 33 and 34.—Ditto. Length of spindle 7·0 μ.

Fig. 35.—Ditto. Length of spindle 7·5 μ.

Figs. 36 and 37.—Ditto. Length of spindle 7·5 μ. (Taken from section of cyst shown in fig. 3.)

Fig. 38.—Ditto. Length of spindle 8·75 μ.

Fig. 39.—Ditto. Length of spindle 9·0 μ.

Fig. 40.—Ditto. Length of spindle 10·25 μ. (Taken from section of cyst shown in fig. 6.)

Fig. 41.—Ditto. Length of spindle 10·25 μ. (Taken from section of cyst shown in fig. 5.)

Fig. 42.—Ditto. Length of spindle 11·25 μ. (Taken from section of cyst shown in fig. 5.)

Fig. 43.—Lateral view of secondary spermatocyte cell in the early anaphase, showing divergence of daughter-chromosomes. Length of spindle 8·25 μ. (Taken from section of cyst shown in fig. 3.)

Fig. 44.—Ditto. Length of spindle 8·75 μ. (Taken from section of cyst shown in fig. 4.)

Fig. 45.—Ditto, at a slightly later stage. Length of spindle 10·75 μ. (Taken from section of cyst shown in fig. 4.)

Fig. 46.—Divisions of stage micrometer, 10 μ apart, showing magnification of figs. 1 to 6 inclusive.

Fig. 47.—Ditto, showing magnification of figs. 7 to 45 inclusive.